IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Re:		
	Application of:	Yoshihiro YOSHIHARA
	Serial No.:	10/620,148
	Filed:	July 14, 2003
	For:	**MODEL ANIMALS FOR VISUALIZATION OF NEURAL PATHWAYS**
	Examiner:	Joanne Hama
	Art Unit:	1632

Commissioner for Patents
P.O. Box 1450
Arlington, VA 22313-1450

DECLARATION OF TETSUO NODA UNDER 37 C.F.R. § 1.132

I, Tetsuo Noda, hereby declare that:

1. I received a M.D. degree from Tohoku University School of Medicine in Sendai, Japan in 1980 and a Ph.D. in Microbiology from the Postgraduate School for Medicine of Tohoku University in Sendai, Japan in 1984. My thesis was entitled "Studies on the mechanisms of INF-y induction in human peripheral blood mononuclear cells."

2. From 1990 to 1997, I was a member of and the chief of the Department of Cell Biology of the Cancer Institute in Tokyo, Japan. From 1997 to 2002, I was a professor in the Department of Molecular Genetics of the Tohoku University School of Medicine in Sendai, Japan. From 2002 to the present, I have been Director of the Center for Translational and Advanced Animal Research of the Tohoku University School of Medicine in Sendai, Japan. A copy of my curriculum vitae is attached hereto as Exhibit 1.

3. I submit this declaration in support of a Response to the Office Action dated September 9, 2004 from the U.S. Patent and Trademark Office in connection with the above-identified application.

4. I understand that, in the Office Action dated September 9, 2004 with respect to this patent application, the Examiner rejected claims 1-5 under 35 U.S.C. § 112, first paragraph, on the grounds that the specification does not enable a person skilled in the art to make and use the invention as claimed. With regard to claims 1-3, the Examiner contended that the specification, while being enabling for a transgenic mouse that expresses wheat germ agglutinin (WGA) under the control of the L7 or OMP promoter, does not reasonably provide enablement, without undue experimentation, for any other "transgenic animal" expressing a trans-synaptic protein, for any other trans-synaptic tracer protein, for any other promoter to drive expression in neurons or for any neural cells, other than ones that express trans-synaptic protein, under the control of the L7 or OMP promoter, since the field of transgenic mammals, including the pronuclear injection method of generating transgenic animals, was unpredictable at the time of the instant application in that results obtained with one species would not have been predictive of results that would be obtained for another species.

5. I submit this declaration in order to demonstrate that the same results and effects obtained for a transgenic mouse that expresses wheat germ agglutinin (WGA) under the control of the L7 or OMP promoter could also be obtained using any other transgenic animal with any other trans-synaptic tracer protein and any other promoters. I demonstrate below that the expression of any trans-synaptic tracer protein not only in mice but in other animals could be achieved according to the description in the specification by a person skilled in the art without undue experimentation as of the date when the invention was made.

6. Trans-synaptic tracer proteins, such as plant lectins described in the specification and nontoxic C-terminal fragment of tetanus neurotoxin (TTC), have conventionally been used for visualization of neural pathways in animals. For example, "trans-synaptic tracer" proteins are well known, as described in Yoshihara, *Neuroscience Research*, Vol. 44, pp. 133-40 (2002), and Horn et al., *Experimental Brain Research*, Vol. 81, pp. 353-62 (1990).

7. These trans-synaptic tracer proteins have been injected into a variety of animals having a nervous system, including rodents such as mice, rats, guinea pigs; primates such as monkeys; and other mammals such as rabbits, cats; and birds. The following references describe injection of WGA into animals other than rodents: LaVail et al., Journal of Cell Biology, Vol. 96, pp. 373-81 (February 1983) (injection into bird); Tanaka et al., *Journal of Laryngology and Otology*, Vol. 107, pp. 916-19 (October 1993) (injection into cat); Pons et al., *Journal of Comparative Neurology*, Vol. 248, pp. 313-35 (1986) (injection into Cynomolgus monkey); and Jensen et al., *Brain Research*, Vol. 586, pp. 125-29 (1992) (injection into rabbit).

8. The present invention enables specific expression of a trans-synaptic tracer protein in particular neurons, by operably linking a neuron-specific promoter and a gene encoding the trans-synaptic tracer protein. For example, as shown in the Examples, by ligating a gene encoding WGA downstream of L7 or OMP promoter, the specific expression of WGA in L7 promoter functioning mouse cerebellar Purkinje cell and OMP promoter functioning mouse olfactory nervous system, and the visualization of the neural pathway via transport of WGA from neuron to neuron, can be established.

9. As reported in Horowitz et al., *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 96, pp. 3194-99 (March 1999), Horowitz et al. generated a transgenic mouse in which a gene encoding barley lectin (BL) linked downstream of OMP promoter is introduced, and employed the mouse in experiments for visualizing neural pathways by detecting the BL. Horowitz et al. accomplished olfactory nervous system specific visualization of neural pathways employing BL, similar to that accomplished in the Examples of the present application using WGA. Horowitz et al. demonstrate that the expression of trans-synaptic tracer proteins other than WGA in transgenic animals can provide the same effect.

10. Contrary to the Examiner's reference to the difficulty in the selection of suitable promoters, neuron-specific promoters can function in a variety of host organisms in a similar manner. For example, OMP promoters, which are described in the specification and function specifically to olfactory neuron, were cloned from rats and mice, and are shown to function in

olfactory neuron-specific manner. A transgenic mouse, in which a lacZ gene fused downstream of rat OMP promoter is introduced, expresses lacZ specifically to olfactory neurons, as reported in Forss-Pettera et al., *Neuron*, Vol. 5, pp. 187-97 (August 1990). This result clearly indicates that the rat neuron-specific promoter can function in a similar manner in mice.

11. Mouse-derived L7 promoter can express lacZ in rat, and the expression is specific to cerebellar Purkinje cells similarly as in mice, as described in the Examples in the specification. Similar to OMP promoter, it is possible to express exogenous proteins in a wide range of host organisms employing this neuron-specific promoter. Further, L7 protein, which is naturally expressed by the L7 promoter, has been cloned from mice, rats, and human. The amino acid sequences thereof are highly conserved among species, and the proteins are functionally correlated and regulated so as to express specifically in cerebellar Purkinje cells, as reported in Zhang et al., *Molecular Brain Research*, Vol. 105, pp. 1-10 (2002).

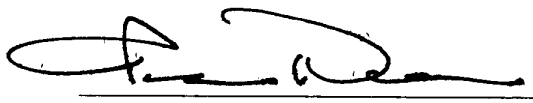
12. The protein Olf-1 is known as a trans-acting regulator for OMP promoter, and the sequence of cis-acting element, to which the Olf-1 binds, has been found in the OMP promoter. It is possible to identify olfactory neuron specific promoters other than OMP and regulating proteins using common computer analysis based on these sequences, as reported in Wang et al., *Molecular and Cellular Biology*, Vol. 13, No. 9, pp. 5805-13 (September 1993).

13. As reported in Maskos et al., *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 99, No. 15, pp. 10120-25 (July 2002), Maskos et al. generate a transgenic mouse in which a gene encoding a GFP-TTC fusion protein linked downstream of rat Calbindin promoter, which function in cerebellar Purkinje cells, is introduced. TTC is a protein known to be a trans-synaptic tracer, as described above by Horn et al. As shown by Maskos et al., the GFP-TTC fusion protein is expressed and visualized in the transgenic mouse, and the expression pattern is the same as in rats, showing not only that a trans-synaptic tracer protein other than WGA can be used in the present invention, but also that a rat neuron-specific promoter can function in mice in the same way.

14. Tabuchi et al., as reported in *Journal of Neuroscience Research*, Vol. 59, pp. 94-99 (2000), demonstrate that the present invention can be applied to other animal species. Tabuchi et al. succeeded in visualizing optic pathways by expressing WGA operably linked via GAL4 to a Rhodopsin promoter (Rd1 promoter) in Dorosophila photoreceptor cells. Of course, various species of transgenic animals, such as rats, pigs, and chicks, were known in the art before the date when the invention was made as described in several publications. For example, transgenic rats could be obtained from suppliers such as Institute of YS New Technology Ltd., Tochigi, Japan or prepared according to Hirabayashi et al., "Transgene expression in mammary glands of newborn rats", Mol. Reprod. Dev. 43(2): 145-149 (1996).

15. Accordingly, I believe that claims 1-3 as amended are fully enabled by the specification as of the date when the invention was made such that it would not require undue experimentation to obtain the results claimed for a transgenic mouse that expresses wheat germ agglutinin (WGA) under the control of the L7 or OMP promoter by using any other transgenic animal with any other trans-synaptic tracer protein and any other promoters.

I hereby declare that I understand the English language and that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issued therefrom.



Tetsuo Noda, M.D., Ph.D.

Dated: Sendai, Japan
January 21, 2005

CURRICULUM VITAE

December 1, 2004

Tetsuo Noda, M.D. ,Ph.D.

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1980-1984 Ph.D.
Dept. of Microbiology
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Thesis: " Studies on the mechanisms of INF-γ induction in human peripheral blood mononuclear cells."

Professional Experience:

1984-85 Postdoctoral Fellow,
National Cancer Institute-Frederick Cancer Res. Facility, NIH, U.S.
Supervisor: Dr. Yoshiaki Ito
research on the transformation and transcription with polyoma virus, the cooperation of tumor antigens

1985-88 Research Associate,
Institute for Virus Research, Kyoto University, Kyoto, Japan
research on the transformation mechanisms by human papilomaviruses (HPV), the identification and isolation

of new type HPV

1988-91 Visiting Scientist,
Whitehead Institute for Biomedical Research,
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1990-1997 Member and Chief,
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